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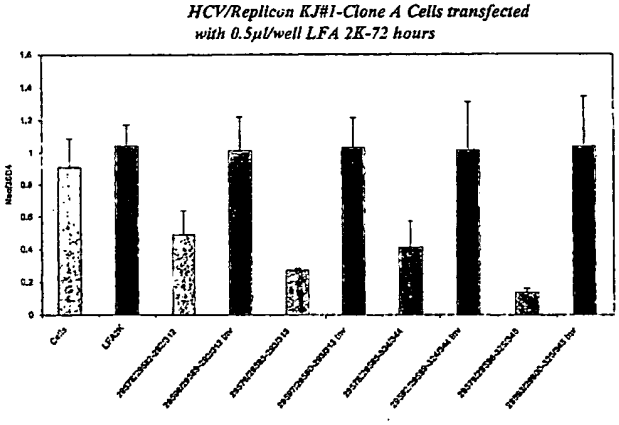
(54) Title: RNA INTERFERENCE MEDIATED INHIBITION OF GENE EXPRESSION USING CHEMICALLY MODIFIED SHORT INTERFERING NUCLEIC ACID



(57) Abstract: The present invention concerns methods and reagents useful in modulating gene expression in a variety of applications, including use in therapeutic, diagnostic, target validation, and genomic discovery applications. Specifically, the invention relates to synthetic chemically modified small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against target nucleic acid sequences. The small nucleic acid molecules are useful in the treatment of any disease or condition that responds to modulation of gene expression or activity in a cell, tissue, or organism.

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CLAIMS

What we claim is:

1. A double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a target gene, wherein said siNA molecule comprises no ribonucleotides and each strand of said double-stranded siNA comprises about 21 nucleotides.

2. The siNA molecule of claim 1, wherein one of the strands of said double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the target gene, and wherein the second strand of said double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of the target gene.

3. The siNA molecule of claim 2, wherein each strand of the siNA molecule comprises about 19 to about 23 nucleotides, and wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand.

4. The siNA molecule of claim 1, wherein said siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the target gene, and wherein said siNA further comprises a sense region, wherein said sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of said target gene.

5. The siNA molecule of claim 4, wherein said antisense region and said sense region each comprise about 19 to about 23 nucleotides, and wherein said antisense region comprises at least about 19 nucleotides that are complementary to nucleotides of the sense region.

6. The siNA molecule of claim 1, wherein said siNA molecule comprises a sense region and an antisense region and wherein said antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of RNA encoded by the target gene and said sense region comprises a nucleotide sequence that is complementary to said antisense region.

7. The siNA molecule of claim 6, wherein said siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of said siNA molecule.

8. The siNA molecule of claim 6, wherein said sense region is connected to the antisense region via a linker molecule.

9. The siNA molecule of claim 8, wherein said linker molecule is a polynucleotide linker.

10. The siNA molecule of claim 8, wherein said linker molecule is a non-nucleotide linker.

11. The siNA molecule of claim 6, wherein pyrimidine nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides and purine nucleotides in the sense region are2'-deoxy purine nucleotides.

12. The siNA molecule of claim 6, wherein the pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides.

13. The siNA molecule of claim 7, wherein the fragment comprising said sense region includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the fragment comprising said sense region.

14. The siNA molecule of claim 13, wherein said terminal cap moiety is an inverted deoxy abasic moiety.

15. The siNA molecule of claim 6, wherein the pyrimidine nucleotides of said antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides of said antisense region are 2'-O-methyl purine nucleotides.

16. The siNA molecule of claim 6, wherein the pyrimidine nucleotides present in said antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein the purine nucleotides present in said antisense region comprise 2'-deoxy- purine nucleotides.

17. The siNA molecule of claim 15, wherein said antisense region comprises a phosphorothioate internucleotide linkage at the 3' end of said antisense region.

18. The siNA molecule of claim 6, wherein said antisense region comprises a glyceryl modification at the 3' end of said antisense region.

19. The siNA molecule of claim 7, wherein each of the two fragments of said siNA molecule comprise 21 nucleotides.

20. The siNA molecule of claim 17, wherein about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule and wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule.

21. The siNA molecule of claim 20, wherein each of the two 3' terminal nucleotides of each fragment of the siNA molecule are 2'-deoxy-pyrimidines.

22. The siNA molecule of claim 21, wherein the 2'-deoxy-pyrimidine is 2'-deoxy-thymidine.

23. The siNA molecule of claim 19, wherein all 21 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule.

24. The siNA molecule of claim 19, wherein about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the target gene.

25. The siNA molecule of claim 19, wherein 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the target gene.

26. The siNA molecule of claim 7, wherein the 5'-end of the fragment comprising said antisense region optionally includes a phosphate group.

27. The siNA molecule of claim 1, wherein said target gene is a mammalian gene.

28. The siNA molecule of claim 1, wherein said target gene is a plant gene.

29. The siNA molecule of claim 1, wherein said target gene is a bacterial gene.

30. The siNA molecule of claim 1, wherein said target gene is a fungal gene.

31. The siNA molecule of claim 1, wherein said target gene is a viral gene.

32. The siNA molecule of claim 27, wherein said mammalian gene is a human gene.

33. A double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a target RNA sequence, wherein said siNA molecule comprises no ribonucleotides and wherein each strand of said double-stranded siNA molecule comprises about 21 nucleotides g.

34. The siNA molecule of claim 33, wherein said target RNA sequence is encoded by a viral genome.

35. The siNA molecule of claim 33, wherein said target RNA sequence is encoded by a bacterial gene.

36. The siNA molecule of claim 33, wherein said target RNA sequence is encoded by a mammalian gene.

37. The siNA molecule of claim 36, wherein said mammalian gene is a human gene.

38. The siNA molecule of claim 33, wherein said target RNA sequence is encoded by a plant gene.

39. A double-stranded short interfering nucleic acid (siNA) molecule that inhibits the replication of a virus, wherein said siNA molecule comprises no ribonucleotides and each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

40. The siNA molecule of claim 39, wherein said virus is a mammalian virus.

41. The siNA molecule of claim 39, wherein said virus is a plant virus.

42. The siNA molecule of claim 40, wherein said mammalian virus is hepatitis C virus.

43. The siNA molecule of claim 40, wherein said mammalian virus is human immunodeficiency virus.

44. The siNA molecule of claim 40, wherein said mammalian virus is hepatitis B virus.

45. The siNA molecule of claim 40, wherein said mammalian virus is herpes simplex virus.

46. The siNA molecule of claim 40, wherein said mammalian virus is cytomegalovirus.

47. The siNA molecule of claim 40, wherein said mammalian virus is human papilloma virus.

48. The siNA molecule of claim 40, wherein said mammalian virus is respiratory syncytial virus.

49. The siNA molecule of claim 40, wherein said mammalian virus is influenza virus.

50. A double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a target gene, wherein said siNA molecule does not require the presence of a ribonucleotide within the siNA molecule for said inhibition of expression of a target gene and wherein each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

51. The siNA molecule of claim 50, wherein said target gene is a mammalian gene.

52. The siNA molecule of claim 50, wherein said target gene is a plant gene.

53. The siNA molecule of claim 50, wherein said target gene is a bacterial gene.

54. The siNA molecule of claim 50, wherein said target gene is a fungal gene.

55. The siNA molecule of claim 50, wherein said target gene is a viral gene.

56. The siNA molecule of claim 51, wherein said mammalian gene is a human gene.

57. A double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a target gene by mediating RNA interference (RNAi) process, wherein said siNA molecule comprises no ribonucleotides and wherein each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

58. The siNA molecule of claim 57, wherein said target gene is encoded by a viral genome.

59. The siNA molecule of claim 57, wherein said target gene is a bacterial gene.

60. The siNA molecule of claim 57, wherein said target gene is a mammalian gene.

61. The siNA molecule of claim 60, wherein said mammalian gene is a human gene.

62. The siNA molecule of claim 57, wherein said target gene is a plant gene.

63. A double-stranded short interfering nucleic acid (siNA) molecule that inhibits the replication of a virus, wherein said siNA molecule does not require the presence of a ribonucleotide within the siNA molecule for said inhibition of replication of a virus and each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

64. The siNA molecule of claim 63, wherein said virus is a mammalian virus.

65. The siNA molecule of claim 63, wherein said virus is a plant virus.

66. The siNA molecule of claim 64, wherein said mammalian virus is hepatitis C virus.

67. The siNA molecule of claim 64, wherein said mammalian virus is human immunodeficiency virus.

68. The siNA molecule of claim 64, wherein said mammalian virus is hepatitis B virus.

69. The siNA molecule of claim 64, wherein said mammalian virus is herpes simplex virus.

70. The siNA molecule of claim 64, wherein said mammalian virus is cytomegalovirus.

71. The siNA molecule of claim 64, wherein said mammalian virus is human papilloma virus.

72. The siNA molecule of claim 64, wherein said mammalian virus is respiratory syncytial virus.

73. The siNA molecule of claim 64, wherein said mammalian virus is influenza virus.

74. A pharmaceutical composition comprising the siNA molecule of any of claims 1, 33, 39, 50, 57 and 63 in an acceptable carrier or diluent.

75. Medicament comprising a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a target gene, wherein said siNA molecule comprises no ribonucleotides and each strand of said double-stranded siNA comprises about 21 nucleotides.

76. Medicament according to claim 75, wherein one of the strands of said double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the target gene, and wherein the second strand of said double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of the target gene.

77. Medicament according to claim 76, wherein each said strand of the siNA molecule comprises about 19 to about 23 nucleotides, and wherein each said strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand.

78. Medicament according to claim 75, wherein said siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the target gene, and wherein said siNA further comprises a sense region, wherein said sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of said target gene.

79. Medicament according to claim 78, wherein said antisense region and said sense region each comprise about 19 to about 23 nucleotides, and wherein said antisense region comprises at least about 19 nucleotides that are complementary to nucleotides of the sense region.

80. Medicament according to claim 75, wherein said siNA molecule comprises a sense region and an antisense region and wherein said antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of RNA encoded by the target gene and said sense region comprises a nucleotide sequence that is complementary to said antisense region.

81. Medicament according to claim 80, wherein said siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of said siNA molecule.

82. Medicament according to claim 80, wherein said sense region is connected to the antisense region via a linker molecule.

83. Medicament according to claim 82, wherein said linker molecule is a polynucleotide linker.

84. Medicament according to claim 82, wherein said linker molecule is a non-nucleotide linker.

85. Medicament according to claim 80, wherein pyrimidine nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides and purine nucleotides in the sense region are2'-deoxy purine nucleotides.

86. Medicament according to claim 80, wherein the pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides.

87. Medicament according to claim 81, wherein the fragment comprising said sense region includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the fragment comprising said sense region.

88. Medicament according to claim 87, wherein said terminal cap moiety is an inverted deoxy abasic moiety.

89. Medicament according to claim 80, wherein the pyrimidine nucleotides of said antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides of said antisense region are 2'-O-methyl purine nucleotides.

90. Medicament according to claim 80, wherein the pyrimidine nucleotides present in said antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and wherein the purine nucleotides present in said antisense region comprise 2'-deoxy- purine nucleotides.

91. Medicament according to claim 89, wherein said antisense region comprises a phosphorothioate internucleotide linkage at the 3' end of said antisense region.

92. Medicament according to claim 80, wherein said antisense region comprises a glyceryl modification at the 3' end of said antisense region.

93. Medicament according to 81, wherein each of the two fragments of said siNA molecule comprise 21 nucleotides.

94. Medicament according to claim 91, wherein about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule and wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule.

95. Medicament according to claim 94, wherein each of the two 3' terminal nucleotides of each fragment of the siNA molecule are 2'-deoxy-pyrimidines.

96. Medicament according to claim 95, wherein said 2'-deoxy-pyrimidine is 2'-deoxy-thymidine.

97. Medicament according to claim 93, wherein all 21 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule.

98. Medicament according to claim 93, wherein about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the target gene.

99. Medicament according to claim 93, wherein 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the target gene.

100. Medicament according to claim 81, wherein the 5'-end of the fragment comprising said antisense region optionally includes a phosphate group.

101. Medicament according to claim 75, wherein said target gene is a mammalian gene.

102. Medicament according to claim 75, wherein said target gene is a plant gene.

103. Medicament according to claim 75, wherein said target gene is a bacterial gene.

104. Medicament according to claim 75, wherein said target gene is a fungal gene.

105. Medicament according to claim 75, wherein said target gene is a viral gene.

106. Medicament according to claim 101, wherein said mammalian gene is a human gene.

107. Medicament comprising a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a target RNA sequence, wherein said siNA molecule comprises no ribonucleotides and wherein each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

108. Medicament according to claim 107, wherein said target RNA sequence is encoded by a viral genome.

109. Medicament according to claim 107, wherein said target RNA sequence is encoded by a bacterial gene.

110. Medicament according to claim 107, wherein said target RNA sequence is encoded by a mammalian gene.

111. Medicament according to claim 110, wherein said mammalian gene is a human gene.

112. Medicament according to claim 107, wherein said target RNA sequence is encoded by a plant gene.

113. Medicament comprising a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the replication of a virus, wherein said siNA molecule comprises no ribonucleotides and each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

114. Medicament according to claim 113, wherein said virus is a mammalian virus.

115. Medicament according to claim 113, wherein said virus is a plant virus.

116. Medicament according to claim 114, wherein said mammalian virus is hepatitis C virus.

117. Medicament according to claim 114, wherein said mammalian virus is human immunodeficiency virus.

118. Medicament according to claim 114, wherein said mammalian virus is hepatitis B virus.

119. Medicament according to claim 114, wherein said mammalian virus is herpes simplex virus.

120. Medicament according to claim 114, wherein said mammalian virus is cytomegalovirus.

121. Medicament according to claim 114, wherein said mammalian virus is human papilloma virus.

122. Medicament according to claim 114, wherein said mammalian virus is respiratory syncytial virus.

123. Medicament according to claim 114, wherein said mammalian virus is influenza virus.

124. Medicament comprising a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a target gene, wherein said siNA molecule does not require the presence of a ribonucleotide within the siNA molecule for said inhibition of expression of a target gene and wherein each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

125. Medicament according to claim 124, wherein said target gene is a mammalian gene.

126. Medicament according to claim 124, wherein said target gene is a plant gene.

127. Medicament according to claim 124, wherein said target gene is a bacterial gene.

128. Medicament according to claim 124, wherein said target gene is a fungal gene.

129. Medicament according to claim 124, wherein said target gene is a viral gene.

130. Medicament according to claim 125, wherein said mammalian gene is a human gene.

131. Medicament comprising a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a target gene by mediating RNA interference (RNAi) process, wherein said siNA molecule comprises no ribonucleotides and wherein each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

132. Medicament comprising a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the replication of a virus, wherein said siNA molecule does not require the presence of a ribonucleotide within the siNA molecule for said inhibition of replication of a virus and each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

133. Medicament according to claim 132, wherein said target gene is encoded by a viral genome.

134. Medicament according to claim 132, wherein said target gene is a bacterial gene.

135. Medicament according to claim 132, wherein said target gene is a mammalian gene.

136. Medicament according to claim 135, wherein said mammalian gene is a human gene.

137. Medicament according to claim 132, wherein said target gene is a plant gene.

138. Active ingredient comprising a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a target gene, wherein said siNA molecule comprises no ribonucleotides and each strand of said double-stranded siNA comprises about 21 nucleotides.

139. Active ingredient comprising a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a target RNA sequence, wherein said siNA molecule comprises no ribonucleotides and wherein each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

140. Active ingredient comprising a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the replication of a virus, wherein said siNA molecule comprises no ribonucleotides and each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

141. Active ingredient comprising a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a target gene, wherein said siNA molecule does not require the presence of a ribonucleotide within the siNA molecule for said inhibition of expression of a target gene and wherein each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

142. Active ingredient comprising a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a target gene by mediating RNA interference (RNAi) process, wherein said siNA molecule comprises no ribonucleotides and wherein each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

143. Active ingredient comprising a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the replication of a virus, wherein said siNA molecule does not require the presence of a ribonucleotide within the siNA molecule for said inhibition of replication of a virus and each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

144. Use of a double-stranded short interfering nucleic acid (siNA) molecule to down-regulate expression of a target gene, wherein said siNA molecule comprises no ribonucleotides and each strand of said double-stranded siNA comprises about 21 nucleotides.

145. Use of a double-stranded short interfering nucleic acid (siNA) molecule to inhibit the expression of a target RNA sequence, wherein said siNA molecule comprises no ribonucleotides and wherein each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

146. Use of a double-stranded short interfering nucleic acid (siNA) molecule to inhibit the replication of a virus, wherein said siNA molecule comprises no ribonucleotides and each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

147. Use of a double-stranded short interfering nucleic acid (siNA) molecule to inhibit the expression of a target gene, wherein said siNA molecule does not require the presence of a ribonucleotide within the siNA molecule for said inhibition of expression of a target gene and wherein each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

148. Use of a double-stranded short interfering nucleic acid (siNA) molecule to inhibit the expression of a target gene by mediating RNA interference (RNAi) process, wherein said siNA molecule comprises no ribonucleotides and wherein each strand of said double-stranded siNA molecule comprises about 21 nucleotides.

149. Use of a double-stranded short interfering nucleic acid (siNA) molecule to inhibit the replication of a virus, wherein said siNA molecule does not require the presence of a ribonucleotide within the siNA molecule for said inhibition of replication of a virus and each strand of said double-stranded siNA molecule comprises about 21 nucleotides.